Application of Isotope Dilution for the Determination of Thorium in Biological Samples by Inductively Coupled Plasma Mass Spectrometry

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The applicability of isotope dilution-inductively coupled plasma mass spectrometry (ID-ICP-MS) was examined for Th in biological samples. A naturally occurring isotope of Th (Th-230) was used as the spiking isotope (called "tracer" in the text). The concentration of Th-230 in the final sample solution was about 50–60 pg/ml; an isotope ratio of 232/230 could be measured with a relative standard deviation of less than 2%. The error magnification depended on the amount of Th-232 being concomitant with the Th-230. Though it was shown that one ng of Th-232 could be determined with reasonable precision with a tracer of the present purity, more care should be taken to reduce any source of systematic error.

Keywords Isotope dilution analysis, inductively coupled plasma mass spectrometry, thorium, biological sample

Inductively coupled plasma mass spectrometry (ICP-MS) has many attractive features as a tool for both trace and ultratrace analyses: e.g. ease of sample introduction as well as the rapid measurement time of several minutes to obtain a normal multielement analytical results. Naturally, it is the determination of the isotopic base, which makes isotope dilution (ID) analysis available by ICP-MS. Considerable interest and attention has been paid to this promising methodology, ID-ICP-MS.¹⁻¹² As many investigators have noted, there are two major advantages. Firstly, troublesome problems of ICP-MS, such as drift and matrix effects, are eliminated by applying the ID methodology. Secondly, in cases where chemical separation or preconcentration is involved, no correction is required for analyte recovery. In spite of the wide applicability of ID-ICP-MS, no work has been reported concerning the determination of Th in biological samples.

We have applied ICP-MS to measurements of long-lived radionuclides in the environment.¹³ Also, we now have an increased appreciation that accurate and sensitive analytical techniques are necessary in order to determine such nuclides in environmental samples. This is especially true in the determination of Th and U in human bone samples, where we encountered difficulties; this is due to large amounts of matrix elements, mainly

Ca and P.¹⁴ The reported Th and U concentrations in human bones are widely scattered; from sub to several ng/g ash.¹⁵⁻¹⁹ To avoid blockage of the ICP-MS sampler, the concentration of ash in the sample solution should therefore not exceed 0.8–1 mg ash/ml, so that dilution gives an analyte concentration of sub to several pg/ml. The present sensitivity (response) of the commercial quadrupole ICP-mass spectrometer is in the range of 1–10 counts per second (cps) per ng/ml analyte solution, and the detection limits are from sub to several pg/ml, which are not necessarily sufficient in this instance.

In order to correctly measure Th and U in bone samples, they should be chemically separated from the matrix and concentrated so as to be at least several tens pg/ml; this requires yield monitoring. As mentioned above, if isotopes are available in the ID analysis, it is attractive and advantageous. Thus, an alternative selection (ID-ICP-MS) was studied for Th in biological samples using a natural isotope (Th-230) as the spiking one, and also using biological standard and certified reference materials (SRMs and CRMs).

Experimental

Samples

Samples were biological SRMs and CRMs from the U.S. National Institute of Standards and Technology

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(NIST) and Japan National Institute for Environmental Studies (NIES)^{20,21}, respectively. After drying, about 100–300 mg of the sample was directly weighed in the PTFE decomposition vessel for sample digestion with acids (San-ai Kagaku, Nagoya, Japan). The vessel was covered by a conductible rubber bag during weighing in order to avoid any scattering of the sample powder by static electricity. The vessel was heated on a hot plate while adding HNO₃ and H₂O₂. It was then heated in an electric oven with an iron jacket while adding HClO₄ and HF in order to decompose residual organic and silicious matter. The spike solution of Th-230 (to avoid any confusion with the sample, hereafter called "tracer") was added prior to acid digestion in the oven. The thus-obtained clear solution was evaporated to a syrup, and HNO₃ was added. The solution was then evaporated and the residue dissolved in 10 ml of 8 M HNO₃. Thorium was separated by the use of an anion exchange method. The resin used was BIO-RAD AG1×4 50–100 mesh (1 ml). After passing the sample solution, the resin column was washed using 10 ml of 8 M HNO₃ and Th was elute by 10 ml of 6 M HCl. The eluate was evaporated off and the residue dissolved in 10 ml of 10% (1.6 M) HNO₃. At this step, samples which were found to contain an insoluble residue were treated by adding H₂O₂ and HClO₄ (several drops), and then dissolved again in 10% HNO₃.

The reagents were all of high purity (Tama Pure AA-100, Tama Chemical, Kawasaki, Japan). The pure water was of high resistivity (more than 18 MΩ cm), obtained from Barnstead Nano Pure (Barnstead, Boston, MA, USA). The PTFE and glass wares used were cleaned by boiling or soaking in an HNO₃ solution.

Calculation

Since the content of Th-230 in the biological samples may be neglected, the Th-232 content in the samples (*X*) was calculated using

$$X = (R_m - R_t) \times T. \quad (1)$$

Here, $R_m = [232]_m / [230]_m$, $R_t = [232]_t / [230]_t$ and $T = [230]_t / [230]_m$ and [232] and [230] indicate the concentrations of Th-230 and Th-232 in molar units, respectively. Subscripts m and t, respectively, indicate the mixture and tracer. The average isotope ratio was obtained from 5 sets of ratios calculated from the background subtracted count rate. The standard deviation of the *X* (*s_X*) was estimated using

$$s_X = X \times \sqrt{(s_{R_m}^2 + s_{R_t}^2) / (R_m - R_t)^2 + (s_T / T)^2}; \quad (2)$$

where *s_{R_m}*, *s_{R_t}* and *s_T* are the standard deviations of *R_m*, *R_t* and *T*, respectively.

Thorium-230 tracer

The Th-230 tracer solution was prepared from UO₂·(NO₃)₂·6H₂O kept for a long time in the laboratory. Thorium-230 grows from its parent (U-234) by radio-decay, and accumulates in the reagent with time. It has

been calculated that 1 g of natural uranium (corresponding to about 2 g of UO₂·(NO₃)₂·6H₂O) contains about 1 ng of Th-230 ten years after production. Uranyl nitrate contains natural Th-232 as an impurity. As described later, if the content is sufficiently low, separated Th-230 can be used as a tracer in ID analysis.

Thorium-230 in UO₂·(NO₃)₂·6H₂O was separated as follows. Uranyl nitrate was dissolved in 8 M HNO₃; the solution was then passed through an anion exchange resin column (Dowex 1×8, 100–200 mesh). The column was washed by 8 M HNO₃ and the Th adsorbed on the column was eluted by 6 M HCl. The eluate was added with HNO₃ and HClO₄. It was then heated in order to decompose the organic matter, and finally dried up. The obtained residue was dissolved in an HNO₃ solution. Tracers were prepared from several UO₂·(NO₃)₂ products made by different makers; their purity (contents of Th-232) was tested by using α-spectrometry and ICP-MS. A few of the tracers tested seemed to have sufficient purity for our purpose.

The one having the best purity (the lowest molar ratio of Th-232 and 230 (232/230)) was used in our study. The ratio was about 7. About 600 pg of Th-230 was added to the samples, giving about 60 pg/ml in the final sample solution (10 ml) subjected to the ICP-MS measurement. The content of Th-230 in the tracer was determined by an inverse ID analysis upon adding 10.0 ng of Th-232. The amount of Th-230 in this Th-232 standard was negligibly small, which was confirmed by α-spectrometry.

Measurement

The measurement was carried out on a VG Elemental ICP-mass spectrometer (PlasmaQuad PQ2 Plus) installed at Marubun Co., Minamisuna, Koto-ku, Tokyo.

Since the peak shape obtained by a quadrupole mass spectrometer is triangular, and the present tracer (Th-230) concentration was very low, in order to obtain a higher counting rate, the "peak jumping mode", instead

Table 1 Operating conditions of the ICP-mass spectrometer

ICP conditions	
RF power (kW)	1.2
Coolant gas flow (l/min)	13
Auxiliary gas flow (l/min)	0.5
Carrier gas flow (l/min)	1.1
Soln. uptake rate (ml/min)	0.5
Interface conditions	
Load coil to aperture (mm)	7
Aperture diameter (mm)	1
Data acquisition	
Mass range (a.m.u.)	229–233.7
Points per peak (ch)	5
Dac step between points	5
Peak jump dwell time (ms/ch)	10.24
Number of sweeps	1000
Approx. anal. time (min/measurement)	1

of the scanning mode, was used in the present study. In the "peak jumping mode" odd numbers of points were fixed around the peak center, the region of which is narrower than the unit mass. With the same measurement time, a higher counting rate is obtainable, compared with that of the scanning mode.

Table 1 summarizes the operating parameters of the device. Here, the digital analog conversion (dac in Table 1) was 0.03 a.m.u., meaning that the following was measured:

$$5 \text{ points per peak} \times 5 \text{ steps} \times 0.03 \text{ a.m.u.} = 0.75 \text{ a.m.u.}$$

Results and Discussion

Figure 1 shows the ICP-mass spectrum of Th isotopes in the tracer solution. The sensitivity of the device was about 5000 cps per ng/ml solution of Bi. Table 2 gives the raw results concerning measurements of a *Chlorella* solution at a Th-230 concentration of about 60 pg/ml. The figures of the count given are "area cps"; multiplying the present measurement time for the unit mass (about 50 s) thus gives the total counts for Th-230 to be beyond 10000. This count gives less than one % of one Poisson standard deviation, which is the intrinsic fluctuation in ion counting. The average background count rates were 12.6 and 15.6 cps for Th-230 and 232, respectively. The relative standard deviation (RSD) of the 232/230 ratio (background corrected) was about 1.9% in this

Fig. 1 ICP-mass spectrum of the Th-230 tracer used.

Table 2 Count rates of Th-230 and 232 obtained for *Chlorella* (area cps)

No.	Th-230	Th-232	Ratio (Th-232/230)
1	223.4	1928.4	8.632
2	213.4	1934.4	9.065
3	216.4	1925.4	8.897
4	218.4	1924.4	8.811
5	224.4	1952.4	8.701
Ave.			8.821 ± 0.170 ^a

Blank (average of five replicates) subtracted.

a. One standard deviation (RSD: 1.93%).

instance. This was the maximum RSD of the R_m measured, which seemed to be sufficient for an ID analysis of routine samples. The lowest RSD obtained was 0.4% in this work. Without matrices, a highly stable signal can be expected. Almost no noticeable drift of the signal intensity was observed in the secular measurement.

One important problem in an ID analysis concerns mass discrimination effects in measurements. However, as Longerich has pointed out¹, within a short time range, the isotope ratio in the tracer, the sample (no need in the present instance) and the mixture, can be measured, so that the mass discrimination effect may be canceled out. Also, the "true" value of the ratio in the tracer (R_t) is not necessary for the same reason. The presence of matrix elements influences the measurement, and mass discrimination may be induced.²² Although the effect seems to be small between the isotopes of a given element, proper dilution of the sample solution or isolation of the analyte seems to be favorable.

In a general ID analysis, the ratio of a given isotope to another one of the analyte element in the sample must be involved. Since the activity concentration of Th-230 in biological samples seems to be as low as that of Th-232, resulting in $10^4 - 10^5$ (232/230) atoms, this can be considered to be negligible and below the detection limits under the present conditions. This results in a simpler calculation than when using the more general equation of ID analysis, Eq. (1).

The following discussion shows the limitation of the present method; i.e. the amount of Th that can be determined with sufficient precision. As given in Eq. (2), the entire precision of the result depends on the precisions of R_m , R_t and T . R_t was determined in the same manner as was samples at a concentration 3-times as high as the samples, obtaining results with 0.4% RSD. The T was determined with an RSD of 1.5% by an inverse ID analysis. To obtain sufficient precision, only magnification of the error by varying R_m should be considered. The relative standard deviation (s_x/X) can be expressed in terms of those of R_m and R_t ; rearranging Eq. (2) gives

$$\sqrt{\frac{(s_{R_m}^2/R_m^2) \times R_m^2 + (s_{R_t}^2/R_t^2) \times R_t^2}{(R_m - R_t)^2} + \left(\frac{s_T}{T}\right)^2} \quad (3)$$

If the RSD of the R_m and R_t is assumed to maintain a constant value (a) independent of the concentrations and the second term of Eq. (3) could be small enough to be neglected, the equation would be

$$\sqrt{\frac{a^2 \times (R_m^2 + R_t^2)}{(R_m - R_t)^2}} \quad (4)$$

Here, considering the relation between R_m and R_t ,

$$\begin{aligned} R_m &= \frac{[232]_m}{[230]_m} = \frac{Y \times ([232]_s + [232]_t)}{Y \times [230]_t} \\ &= \frac{n \times [232]_t}{[230]_t} = n \times R_t \end{aligned} \quad (5)$$

$$\text{i.e. } R_m = n \times R_s, \quad n > 1. \quad (6)$$

Y is the chemical yield and subscript s indicates the sample. The RSD of X is therefore expressed in terms of n :

$$s_x/X = \{a/(n-1)\} \times \sqrt{n^2+1}, \quad n > 1. \quad (7)$$

Graphical expressions of Eq. (7) with various (a) are depicted in Fig. 2. As can be clearly seen, the reduction of (a) considerably decreases in the fluctuation of the final result for low n . It is intuitively known that as the content of Th-232 in the tracer decreases (the purity of the tracer is higher) a smaller amount of Th-232 in the

Fig. 2 Error magnification in the result with various " a "; the solid curves indicate the calculated values (graphical expressions of Eq. (7)) and the cross marks indicate n and the RSD values obtained for actual samples, following the curves of $a=1$ and 2% .

sample can be determined with greater precision; this is quantitatively described by Eq. (7). The cross mark plotted in the figure indicates the RSD (involving all factors) of X and n for the samples, following the curve of $a=1$ and 2% .

The requirement for the RSD of X to be below 10% can be found graphically: $a=0.5\%$, $n > 1.08$; $a=1\%$, $n > 1.16$; $a=2\%$, $n > 1.33$. The present tracer contained about 4200 pg of Th-232. Accordingly, at least about 340 pg of Th-232 must have been contained in the sample when (a) was 0.5% . Similarly, 670 and 1400 pg of Th-232 must be contained in the sample for the case that (a) is 1 and 2% , respectively. The value of n for a given R_m only depends on $[232]_t$, as is shown in Eqs. (5) and (6); lessening the amount of the tracer added can result in greater precision. However, since at lower concentrations of Th-230 (a) may become more than 2% , the relation may represent a trade-off. When n is greater than about 50 , the value of Eq. (7) becomes closer to the value of (a). Naturally, the highest precision attainable is for an RSD of $1-2\%$ with the present device capability. Also, more than 100 ng of Th-232 in the sample may result in two problems. One is the "memory effect" and the other is the "signal saturation" (producing a count exceeding the range of the measurement); care must therefore be taken.

The above-mentioned estimation can also be applied to an ID analysis using artificial isotopes as a tracer: for instance, I-129 for iodine, Cs-135 for cesium, U-233 for uranium, etc.

Table 3 shows the results obtained for the samples. For several samples Th could be determined with reasonable precision. However, as mentioned above, since the content of Th-232 in the tracer was not very small, the value for the procedure blank involved about a 40% error, resulting in a deterioration of the precision for some (worse than 20%).

Table 3 Concentrations of Th in the biological SRMs and CRMs, etc. obtained by ID-ICP-MS

Sample	Wt./mg	Concentrations of Th		Literature value ^c /ng g ⁻¹
		Soln. ^a /pg ml ⁻¹	Sample ^b /ng g ⁻¹	
NIST SRMs				
Orchard Leaves	152.9	1263	82.6 ±1.5	64
Human Lung	303.1	1468	48.7 ±1.2	52
NIES CRMs				
Human Plasma	206.1	48.6	1.49±0.34	2.04 (ref. 25)
Mussel	101.3	591.1	58.3 ±2.2	39–46 (Ref. 22)
Chlorella	107.3	101.9	9.49±1.27	8.67 (Ref. 22)
Other Samples				
Thorotrast Liver ^d	2.37×10 ⁻⁶	64.5	(2.71±0.43)×10 ⁸	2.57×10 ⁸ (Ref. 23)
LA5A Diet ^e	100	168.8	16.9 ±1.2	14.9 (Ref. 24)
Procedure blank		20.7		

a. Procedure blank corrected. b. Errors shown are propagated errors based on five replicating measurements. c. Literature values for NIST SRMs are certified, while for NIES CRMs and other samples are reported. d. Liver (ash) obtained from a patient who was administered a radiological contrast media of ThO₂ (Thorotrast). e. One adult's whole meal (three days) collected by using a duplicate portion study in Los Alamos, NM, USA for the study of elemental intake.

The Thorotrast liver was obtained from a patient who had received a radiological contrast medium (of which the main component was ThO_2) called Thorotrast. The Th content in the liver of such patients is surprisingly high compared to that of a normal liver. The present liver was ashed and processed as described elsewhere.²³ The LA5A diet was a diet sample (meals for three days of one of the authors) for studying the elemental intake.²⁴ It was collected by using a duplicate portion study in Los Alamos, NM, USA during his stay. Both samples had been digested by acids, and were stocked as solutions. An appropriate volume of them were subjected to this study. Especially regarding the Thorotrast liver, in order to avoid device memory, a diluted sample solution was used.

Human Lung, Human Plasma, Chlorella, the Thorotrast liver and the LA5A diet gave good results. One explanation for the good agreement between the present and reported values of the latter two may be that equilibrium of Th in the samples and the tracer was complete, since they were solution samples. On the other hand, the analytical values of Orchard Leaves and Mussel were not very good. These samples contained a scattered fine sandy particulate. This may have contained Th, which was suspected to be one reason, because of the small amount used. To obtain good analytical results, the equilibration step also seems to be important. The present procedure for the equilibrium might not be sufficient for some of the samples. The procedure blank was about 210 pg, not very high. However, further efforts to reduce the blank seem to be needed.

It is concluded that the ID analysis for Th in biological samples with the present procedure and tracer could achieve reasonable precision for samples containing ng levels of Th-232. The amount of tracer (Th-230) recommended to be added to the sample is more than several hundred picograms; it is also desirable that the purity of the tracer ($[^{232}\text{Th}]/[^{230}\text{Th}]$) be less than 10. A solution volume of 10 ml is needed for carrying out the replicating isotope ratio measurement five times. It should be noted, however, that one cause of inaccuracy may be a systematic error in the sample treatment and procedures, rather than the precision of the analytical measurement. An inhomogeneity of the analyte in the sample, complete equilibration and other factors which affect the result should be taken into consideration.

The author would like to express their sincere thanks to Dr. R. Hutton, VG Elemental and Dr. T. Shimamura, Kitazato University for their kind discussions concerning the manuscript. Thanks are also due to Dr. Y. Ohmomo of the Institute for Environmental Sciences for his support of the work. The Thorotrast liver was donated by Dr. Y. Ishikawa of the Cancer Institute. Ms. T. Sato and Ms. Y. Sakurai helped in the preparation of the manuscript.

Part of this paper was presented at the Second International

Conference on Plasma Source Mass Spectrometry at the University of Durham in September, 1990.

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(Received March 3, 1992)

(Accepted May 18, 1992)